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RESEARCH ARTICLE

Synthesis and characterization of Silver nanoparticles using *Mentha arvensis* and their Photocatalytic degradation and antimicrobial activities

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ABSTRACT

Plant assisted synthesis of silver nanoparticles (Ag NPs) is designed as ideal approach in the green synthesis. In this protocol, we report a rapid and eco-friendly green synthesis of Ag NPs by using a plant extract of *Mentha arvensis.* The color change from test solution light yellow to dark yellow showing the development of silver nanoparticles (*MALE*-Ag) and conformed by characterization of MALE-Ag NPs by UV-Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR) analysis, High-resolution of scanning electron microscopy. The synthesized MALE-Ag NPs were evaluated for photocatlaytic dye degradation, antimicrobial activity against bacterial strains and antimicrobial cotton fabrics loaded with Ag NPs. Further, the prepared Ag NPs exhibited good photocatalytic efficiency in UV light. Furthermore, the MALE-Ag NPs exhibited good potential antimicrobial activities against selected strains *P. aerouginosa*, *S. aureus, E. aerogenes*, *E. coli*. The *E. aerogenes* showed highest (33 mm) zone of inhibition at 100 µg/mL concentration followed by other strains and the fungi A. *niger*showed 29 mm zone of inhibition. The antimicrobial activity of cotton fabrics loaded with Ag NPs against gram negative *P. aerouginosa*. The results suggest that good antimicrobial activity by the unification of 5gMALE on cotton fabrics. Finally it can be concluded that Ag NPs from *Menthaarvensis* leaf extract showed good photocatalytic dye degradation and potential antimicrobial activitiesinmedical and infection prevention applications.

Keywords: Silver nanoparticles, *Mentha arvenis*, photocatalytic dye degradation, antimicrobial activities, wound dressing.

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CONTENTS

1. Introduction

The point sized nanoparticles consist of their unique properties when compared to their parent particles. These novel properties depend on the size, shape, and morphology of the different nanoparticles. All these properties let this smallest version of particles to interact with the plants, microbes, animals, etc [1-7]. Silver nanoparticles (Ag NPs) have shown stunning bactericidal properties against a vast range of micro-organisms [1-3]. These particles are prepared either to test their morphology or their perspective characteristics. The Ag NPs are used widely is that these do not allow the growth of microorganisms or resist it to a higher extent, it is therefore used in electronics, catalysis, and drugs because of these properties [1-3, 8].

Moreover, the synthesis of gold and Silver Nps consists of using of the number of parts of plants such as flowers, fruits [1] etc. Preparation method defines the morphology, size and the stability of the nanoparticles while the strength of the reducing agent and temperature [1, 4-6] along with the nature/concentration of the solvent also affect it wholly.

Ag NPs make their significant position in all those nanoparticles yet found due to their amazing characteristics of performing as an antimicrobial agent in the solid state too. This characteristic of Ag NPs was recognized much before but it was used only in the oriental medicine, coins.So many reviews exist on fabrication or the characterization of the silver nanoparticles but only a few reporting are available regarding the green synthesis, mechanism of action and the biocidal properties [1-2, 4-5, 8-10]. To know in detail about the biosynthesis of the Ag NPs from the herbal extracts, bacteria or fungi.

To synthesize the Ag NPs in solution there needs to have the reducing agents, stabilizing agent or also known as capping agent and the metal precursor. This method is an excellent alternative for the chemical and physical methods because it is cost effective, can be used for higher yields on a large scale and is eco-friendly. The biological method including the biosynthesis of metal and metal oxide nanoparticles through using some biological agents such as bacteria, fungi, yeast, plant and algal extracts has therefore gained popularity in the region of nanotechnology [1-6].

Syed.*et a*l., [13] it has also reported that the synthesis of Ag NPs could even be done from thermophilic fungus *Humicola sp*. This happens when the modifications or alterations were done to the solution or aqueous medium keeping the room temperature conditions. This is a really simple process to generation of Ag NPs from the extracellular synthesis from *Humicola sp*. Thus, the nanoparticles such formed have been shown on TEM micrograph nicely spread, dispersed and mainly of spherical shape ranging between 5 and 25 nm [14].

Al-Bahrani *et al*. [15] have detailed about the biogenic combination of Ag NPs from tree shellfish mushroom named *Pleurotusostreatus*.

Synthesis of Ag NPs from plants

Beg et al. [16, 17] have revealed a green synthesis of Ag NPs from seed separated out of *Pongamia pinnata*. Karatoprak *et al.* [18] have announced there is a green synthesis of Ag NPs that is found from the therapeutic plant extract *Pelargonium endlicherianum*. Phytomediated combination of circular Ag NPs from *Sambucus nigra* fruit extract has been accounted for by Moldovan *et al*. XRD examination demonstrated them to be crystalline.

Vijay Kumar *et al*. [20] who collected or extracted the Ag NPs from *B. diffusa* plant extricate tried them against three fish bacterial pathogens. Ag NPs manufactured from *P. longum* fruit extricate showed cytotoxic impact against MCF-7 bosom malignant growth cell membrane with an IC50 of 67 µg/mL/24 h [21]. They additionally displayed cancer prevention agent and antimicrobial impacts. Latha *et al.*, [22] have created Ag NPs from leaf remove of *Adathoda vasica* and contemplated their antimicrobial action against *Vibrio parahaemolyticus* in agar medium. The utilization of anti-microbial has made them safe. Under such conditions, Ag NPs have shown up as a compelling cure which spares shrimps from dying. Ag NPs from seed powder remove of *A. Heterophyllus* [23] have additionally shown antibacterial movement against gram positive and gram negative microorganisms. Ag NPs manufactured from leaf remove of *C. thwaitesii* have indicated antibacterial adequacy against *Salmonella typhi, Shigella flexneri* and *Klbsiella pneumoniae* indicating them to be huge. Niraimathi and associates [24] have likewise manufactured Ag NPs from the watery concentrate of *A. sessilis* and demonstrated noteworthy antibacterial and cell reinforcement exercises.

Ag NPs from *Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica,* and *Citrus sinensis* have additionally indicated antibacterial action against *S. aureus, P.aeruginosa, E. coli* [25] and *K. pneumoniae*. Noteworthy action of nanoparticles was watched against *S. aureus* and *E.coli.* Antimicrobial action of colloidal Ag NPs was observed to be higher than the plant separate alone. Nayak *et al*., [26] blended Ag NPs from *Dryopteris crassirhizoma* and observed them be exceptionally successful against *B. cereus* and *P. aeruginosa.* Antibacterial and antifungal functions or actions of Ag NPs were also tried against *B. cereus, S.aureus, C. koseri, P. Aeruginosa* bacteria and *C. albicans* fungus. The recommendations are that Ag NPs infiltrate into the bacterial cell and interface with the thiol, hydroxyl and carboxyl gatherings of the biomolecules present in them, in the end deactivating the regeneration capacities by discharging Ag^+ ions. However, it is not clarified how the Ag^+ ions were delivered.

2. Materials and Methods

Materials

The fresh healthy leaves of *Mentha arvensis* was obtained from nearby raithubazar, Mehidepatanam, Hyderabad, T.S-500007. $AgNO_3$, Methylene blue, Cargo red were purchased from Merk, India.

Fig.1.*Mentha arvensis* plant leaves

2.2 Preparation *M. arvensis* leaf extracts (MALE)

The prepared *M. arvensis* leaf extract (MALE) was prepared by a similar literature procedure was utilized. Briefly, 5 g fresh leaves of *M. Arvensis* were grinded using motar pestle boiled for 10 min in 100 mL ultra-pure water and filtered through Whatmann No. 1 filter paper. The filtered *M. arvensis* extract was named as MALE and stored at - 15 °C until further use.

2.3 Preparation of MALE-Silver nanoparticles (MALE-Ag NPs): MALE-Ag NPs was prepared by rapid and eco-friendly process, firstly, 10 g of leaves altogether washed and finely cut leaves in a 300 mL Erlenmeyer flagon alongside 100 mL of cleaned twofold refined water and afterward heating up the blend for 5 min before at long last tapping it. The concentrate extract was separated through Whatman channel paper no 1 and put away at - 15 °C and could be utilized inside 1 week. The filtrate was treated with watery 1 mM $AgNO_3$ arrangement in an Erlenmeyer jar and brooded at room temperature. Accordingly, a darker yellow arrangement was shaped, showing the development of silver nanoparticles and labelled as MALE-Ag NPs.

Fig. 2. Synthesis of Silver nanoparticles using MALE (MALE-Ag NPs) dark yellow and *Mentha arvensis* leaf extract (MALE) light yellow.

2.4 Characterization techniques

Greenly and eco-friendly synthesized MALE-Ag NPs were characterized by a UV-Visible spectrophotometer, Fourier Transform Infrared spectroscopy (FTIR), Scanning Electron Microscope (TESCAN - Vega TC software) was performed for the phase identification of Ag NPs.

2.5 Photocatalytic dye degradation studies

The stock solution was prepared by adding of 10 mg of Methylene blue dye to 1000 mL of distilled water. About 10 mg of silver nanoparticles synthesized was added to 100 mL of Methylene blue dye solution. A control was also maintained without addition of silver nanoparticles. Before exposing to irradiation, the reaction suspension was well mixed by being magnetically stirred for 30 min to clearly make the equilibrium of the working solution. Afterwards, the dispersion was monitored under the UV-light. At specific time intervals, aliquots of 2-3 mL suspension were filtered and used to evaluate the photocatalytic degradation of dye. Aliquots with a suspension of 2-3 mL were filtered at specific time intervals and used for evaluating photocatalytic color degradation. Percentage of dye degradation was estimated by the following formula: where are the initial concentration of dye solution and the concentration of dye solution after photocatalytic degradation.

2.6 Study of Biological activities

2.6.1 Antibacterial activity:

Antibacterial activity of the synthesized MALE-Ag nanoparticles was analyzed by using the Kirby-Bauer disc diffusion method [27] against different pathogenic bacteria. The medium was sterilized by autoclaving at 121°C for 15 minutes at 15 *psi* pressure and was used to determine the antibacterial activity of *M. arvensis* silver nanoparticles (MALE-Ag NPs). Sterile molten cool (45 °C) agar was poured aseptically into sterile petri plates (15 mL each) and the plates were allowed to solidify at room temperature in sterile condition. After solidification and drying, the plates were seeded with appropriate microorganisms by streaking evenly on to the surface of the medium with a sterile spreader and wells (8 mm diameter) were cut out from the agar plates using a sterile stainless steel bore and filled with 0.1mL of the MALE-Ag NPs solution in respective wells. Tetracycline and double distilled water were used as positive and negative control respectively. The prepared cultures were in Muller Hinton broth and then the plates were incubated at 37 °C for 24 h in the next day the zones of inhibition were measured with a measuring scale. This experiment was carried out in triplicate for their confirmation. The results were read by the presence or absence of zone of inhibition.

2.6.2 Antifungal activity

To determine the antifungal activity of MALE-Ag NPs by using agar disk diffusion method [28]. Sterile molten cool (45 °C) agar was poured aseptically into sterile petri plates (15 mL each) and the plates were allowed to solidify at room temperature in sterile condition. After solidification and drying, the plates were seeded with appropriate microorganisms by spreading evenly on to the surface of the medium with a sterile spreader and wells (6 mm diameter) were cut out from the agar plates using a sterile stainless steel bore and filled with 0.1mL of the each MALE-Ag solution in respective wells. Nystatin and distilled water were used as positive and negative control respectively. Then the plates were incubated at 37 °C for 4 days, the zones of inhibition were measured.

2.6.3. Antimicrobial treatment of cotton fabrics

The antimicrobial efficiency of cotton fabrics and fabrics loaded with MALE-Ag NPs was studied by zone of inhibition method. In this approach *P. aeruginosa* was taken as a bacterial strain. For this study, the cotton fabric and MALE-Ag NPs loaded cotton fabrics cut in to a small

pieces and the stability of antimicrobial activity was observed by zone of inhibition on the culture [29].

3. Results and Discussion

3.1 UV-Visible spectral analysis

Silver nanoparticles were synthesized at different concentrations of leaf extract such as 1–5 mL using 1 mM of $AgNO_3$ were analyzed by UV spectra of Plasmon resonance band observed at 436–446 nm similar to those reported in literature [30].

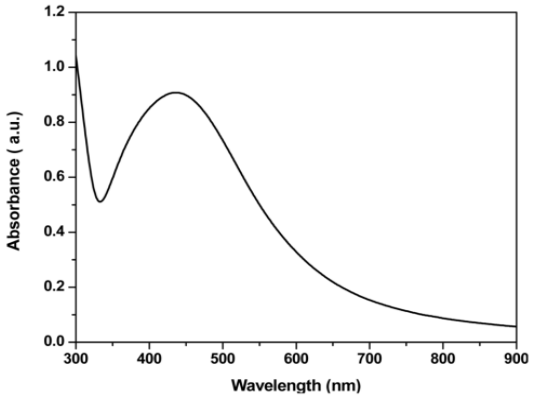

Fig. 3.UV-Visible spectra of MALE-Ag NPs.

3.2 FTIR spectral analysis

The dual role of the plant extract as a reducing and capping agent and presence of some functional groups was confirmed by FTIR analysis of silver nanoparticles. *M. arvensis* showed prominent peaks at 2982 cm^{-1} due to presence of OH stretching, 2088 cm^{-1} due to aliphatic stretching, 1631 cm^{-1} due to carbonyl C=O and 1045 cm^{-1} due to H-C-H deformation extract molecules. The observed peaks are mainly attributed to flavanoids and terpenoids excessively present in plants extract [31]. From FTIR results, it can be concluded that some of the bioorganic compounds from *M. arvensis* leaf extract (MALE) formed a strong coating/capping on the nanoparticles.

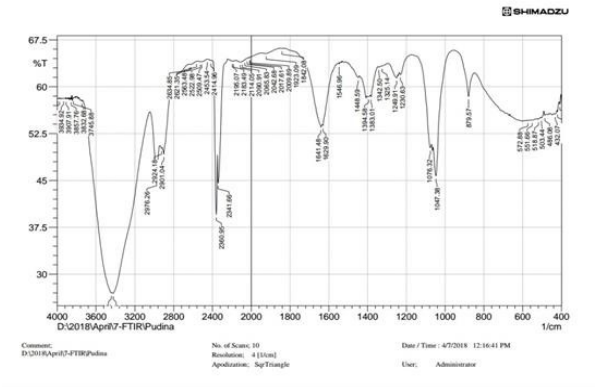

Fig.4. FT-IR spectra of MALE-Ag NPs.

3.3 High-resolution of scanning electron microscopy analysis (HR-SEM): HR- SEM examines each particle, including the aggregate particles, individually; thus, the method is considered to be an absolute measurement of particle size. It can be coupled to image analysis computers for examination of each field for particle distribution. However, the depth of focus is only at 0.5 µm ×1000 and the diffraction effects increase with small particles, which causes blurring at the edges in the determination of particles.

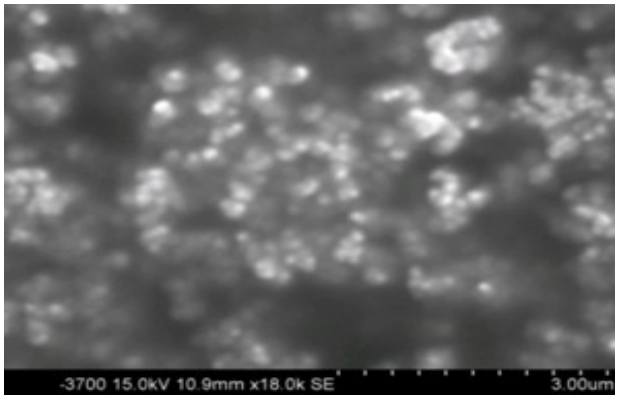

Fig. 5. SEM image of MALE-Ag NPs

3.4 Photo catalytic dye degradation studies

On UV-lamp irradiation with Congo red dye had shown maximum degradation with 44.34 % and minimum degradation 27.66 %. Upon UV-light irradiation with Methylene blue dye solution, MALE-Ag NPs exhibited maximum photo degradation activity with 64.83 % and the minimum percentage degraded with 24.41 % was observed. Therefore, in the presence of light, MALE-Ag NPs with Methylene blue dye showed maximum percentage degradation due to higher absorption property.

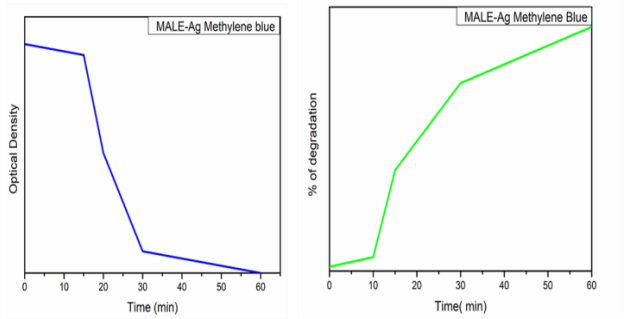

Fig. 6. Photocatalytic degradation kinetics of Methylene blue with MALE-Ag NPs.

Fig. 7. Methylene blue dye color changes with MALE-Ag NPs. (0 minutes to 60 minutes, DW: Distilled Water)

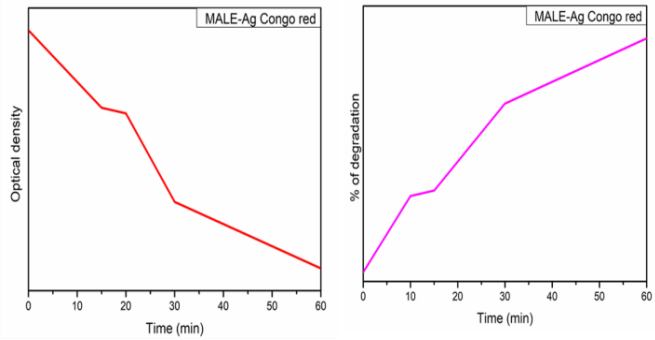

Fig. 8. Photocatalytic degradation kinetics of Congo red with MALE-Ag NPs.

Fig.9. Congo red dye color changes with MALE-Ag NPs. (0 minutes to 60 minutes, DW: Distilled Water)

3.5. Biological activity

3.5.1 Antibacterial activity: In a comparative study of the effect of the Ag NPs in different shapes on the Gram-negative and gram-positive organisms have demonstrated by using Kirby-Bauer disc diffusion method [27]. The stabilized MALE -Ag NPs were tested with bacterial strains including *S. aureus, E. coli* and *P. aeruginosa,* and other strains isolated from human clinical samples such as *P. aeruginosa* (Figure 10). The obtained results showed the zone of inhibition (in mm) at 100 µg/mL of MALE -Ag NPs (SNP) compared with MALE (EXT), Ag NO_3, distilled water (DW) and standard as *streptomycin* (Table 1).

The synthesized MALE -Ag NPs showed good antibacterial activity against *E. aerogenes* and *E. coli* showed highest zone of inhibition (33 mm and 30 mm) and *S. aureus* and *P. aeruginosa* showed nearest zone of inhibition (25 and 22 mm) at 100 µg/mL concentration. However, Ag NO_3, plant extract (MALE), and standard exhibit the least activity when compared to MALE-Ag NPs. Hence, the MALE-Ag NPs exhibits the inhibition of pathogenic bacterial growth in the treatment of infectious diseases caused by bacterial organisms.

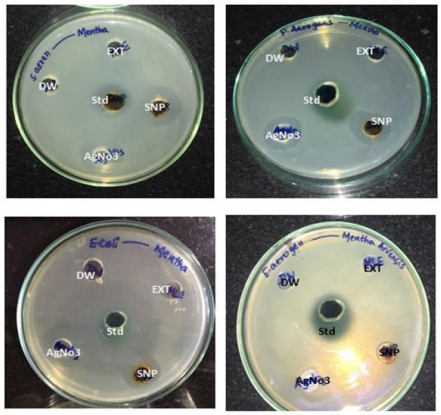

Fig. 10 Representative plats showing antibacterial activity of MALE-AgNps (SNP) against **a)** *S. aureus,* **b)** *P. aeruginosa,* **c)** *E. coli,* and **d)** *E. aerogenes* bacterial strains.

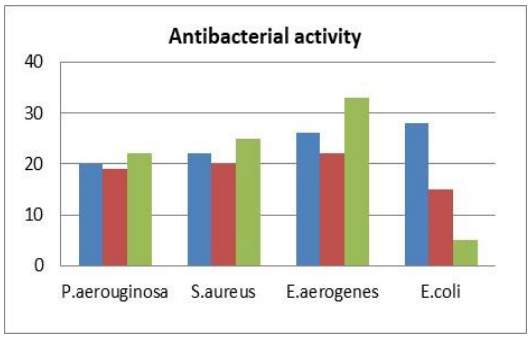

Fig .11. Graph represents the percentage of antibacterial activity of standard (blue), $AgNO_3$ (red), Silver nanoparticles synthesized from aqueous *M. arvensis* leaf extracts (MALE-Ag NPs) (green) at100 µg/mL.

3.5.2 Antifungal activity:

Antifungal activities of plant part extracts against fungi were investigated by the agar disk diffusion method [28]. The MALE-Ag NPs were screened for antifungal activities against *Aspergillus niger* (Figure.12). The five dilution (5, 25, 50, 100, and 250 µg/mL) sets of MALE-Ag NPs (SNP), MALE (EXT), Ag NO_3, distilled water (DW) and standard (*streptomycin*) were prepared in double-distilled water using nutrient agar tubes (Table 2). The zones of growth inhibition around the disks were measured after 18 to 24 hours in incubation at 37 °C for bacteria and 48 to 96 hours for fungi at 28 °C. The sensitivities of the microorganism species to the all samples were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks. The synthesized MALE-Ag NPs showed the good antifungal activity against *Aspergillus niger* showed highest zone of inhibition (29 mm) at 100 µg/mL concentration as compared to standard.

Table-1: Theantibacterial activity of silver nanoparticles(MALE-Ag NPs), Silver Nitrate ($AgNO_3$), Standard *(Streptomycin)*, distilled water, aqueous *Mentha arvensis* leaf extracts (MALE) against organisms and zone of inhibition (in mm) at 100 µg/mL concentration.

Organisms	MALE (EXT)	Distilled water (DW)	Standard (Std) (*Streptomycin*)	$AgNO_3$	MALE-Ag NPs (SNP)
Zone of inhibition in mm					
Pseudomonas aerouginosa	0	0	20	19	22
Staphylococcus aureus	0	0	22	20	25
Enterobacter aerogenes	0	0	26	22	33
Escherichia coli	0	0	28	15	30

Table-2: The antifungal activity of silver nano particles (MALE-Ag NPs), Silver Nitrate ($AgNO_3$), Standard (*Streptomycin)*, Distilled water (DW), aqueous *Mentha arvensis* leaf extract (MALE) against *Aspergillus niger* organism and zone of inhibition (in mm)at 100 µg/mL concentration.

Organisms	MALE (EXT)	Distilled water (DW)	Standard(Std) *(Streptomycin)*	Ag NO_3	MALE-Ag NPs (SNP)
Zone of inhibition in mm					
Aspergillus niger	0	0	19	18	29

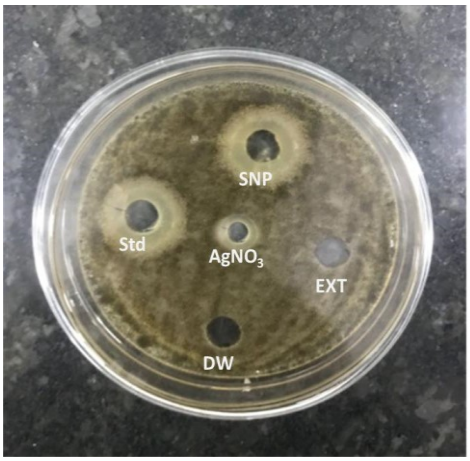

Fig.12. Representative plat showing antifungal activity of MALE-Ag NPs (SNP) against *Aspergillus niger*.

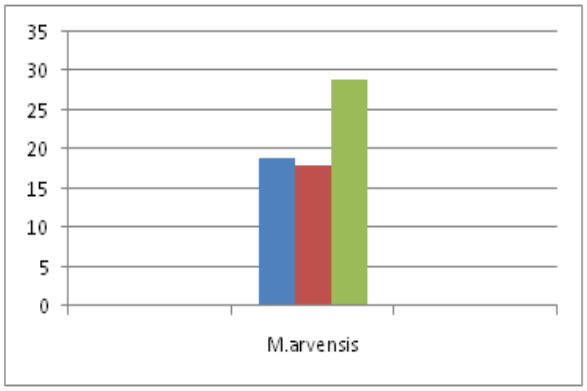

Fig.13. Graph showing antifungal activity of MALE-Ag NPs (green), $AgNO_3$ (red), Standard (blue) at 100 µg/mL.

3.5.3. Antimicrobial efficiency of cotton fabrics

The antimicrobial efficiency of cotton fabrics was tested against gram negative bacterium *Pseudomonas aerouginosa.* Biosynthesized MALE-Ag NPs loaded on cotton fabrics were tested against *P. aerouginosa* and taking as a plain cotton fabric as control (Figure 14). The results obtained that MALE-Ag NPs loaded on cotton fabric exhibited by greater reduction of *P. aerouginosa* growth. Finally the 5g MALE- Ag NPs produces highest antimicrobial effect and release properties as compared with 3g and 4g leaf extracts (MALE). Due to the highest control release properties of this coating utilized for wound healing dressing.

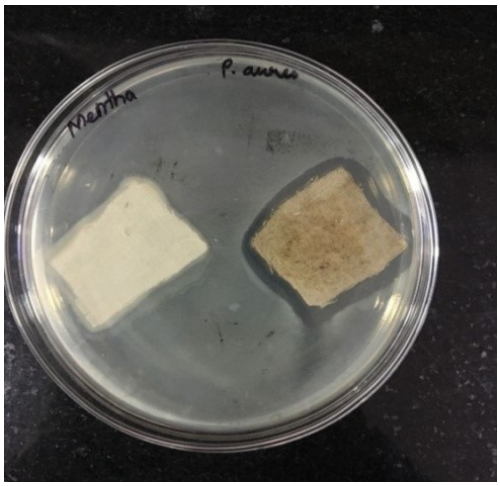

Fig.14. Fig.14. Antimicrobial efficiency of MALE–Ag NPs loaded cotton fabric material.

4. Conclusions

A simple, eco-friendly and rapid synthesis of stable silver nanoparticles using *Mentha arvensis*leaf extract (MALE) in a greener approach. The formation of nanoparticle was identified by the change of colour of MALE and characterized by techniques. Photocatalytic degradation of

dyes (Methylene blue and Cango red) was studied under UV light using MALE-Ag NPs as a catalyst. The MALE-Ag NPs showed good antimicrobial activity against various pathogens. The zone of inhibition proves that the Ag NPs have good antibacterial activity against *Enterobacter aerogenes* followed by *Staphylococcus aureus, Pseudomonas aerouginosa*, and *Escherichia coli*. Further, the synthesized Ag NPs loaded cotton fabrics have exhibited good antimicrobial activity against *P. aerouginosa.* Therefore these fabrics have good exertion in wound healing dressings. The green approach is a valuable application in various fields such as biological and environmental because as they do not involve any toxic reagent.

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